

ASSOCIAZIONE TRA MICROSATELLITI E CARATTERI MORFOLOGICI IN BOVINI DA CARNE

DNA MICROSATELLITES ASSOCIATED WITH MORPHOLOGICAL TRAITS IN BEEF CATTLE

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SUMMARY

The objective of this research was to detect the relationship between DNA microsatellites and morpho-functional traits on Piemontese cattle breed in the attempt to find out major genes and the genomic markers associated with them. A set of 20 microsatellites was analyzed on 47 young double-muscled bulls in a performance test, for which somatic measurements were available. For every allele of each microsatellite, statistical analysis was carried out estimating the significance of the difference between somatic measurements average values of carrying and not carrying subjects. The study revealed numerous microsatellites with alleles significantly linked to positive or negative meat production traits. The most interesting results were represented by INRA 5, INRA 11, INRA 16, INRA 64 and ETH 131. Genomic formulae were examined: this led to originate two furthestmost subpopulations that showed to be significantly distant from each other. The greater homogeneity (*genetic similarities*) was found in the group of subjects with alleles less implicated in meat traits compared to the subjects with positive alleles. The study revealed also that most microsatellites, both in positive and in negative subpopulations, did not respect Hardy-Weinberg proportions, with few microsatellites in clear disequilibrium. Moreover, coefficients for heterozygotes deficiency show the tendency towards homozygosis.

Key words: beef cattle, microsatellites, QTL.

RIASSUNTO

Scopo del presente lavoro è stato identificare le correlazioni tra microsatelliti e caratteri morfo-funzionali nella razza bovina da carne Piemontese, nel tentativo di evidenziare geni ad effetto maggiore e marcatori genomici ad essi associati. È stato analizzato un panel di 20 microsatelliti su 47 giovani tori a groppa doppia in prova di performance per i quali erano disponibili misurazioni somatiche. Per ciascun allele di ciascun microsatellite è stata effettuata l'analisi statistica al fine di saggiare la significatività della differenza tra i valori medi delle misurazioni somatiche di soggetti portatori e non. Lo studio ha rivelato la presenza di numerosi microsatelliti con alleli significativamente associati a caratteri positivi o negativi di produzione della carne. I risultati più interes-

santi sono forniti dai microsatelliti INRA 5, INRA 11, INRA 16, INRA 64 ed ETH 131. L'esame delle formule genomiche ha portato alla creazione di due sottopopolazioni estreme tra loro significativamente distanti. L'omogeneità (*somiglianza genetica*) maggiore è stata riscontrata nel gruppo di soggetti portatori degli alleli meno implicati nei caratteri di produzione della carne se confrontati con soggetti portatori degli alleli positivi per tali caratteri. Lo studio ha rivelato, inoltre che la maggior parte dei microsatelliti, sia nella sottopopolazione dei soggetti positivi che in quella dei negativi, non rispettavano le proporzioni di Hardy-Weinberg e che alcuni microsatelliti manifestavano chiaro disequilibrio. In aggiunta, i coefficienti di difetto degli eterozigoti hanno rivelato la tendenza verso l'omozigosi.

Parole chiave: carne bovina, microsatelliti, QTL.

INTRODUCTION

Meat production aptitude is dependent on the action of numerous genes; it can derive from the limited but combined effects of many genes as well as from the action of major genes, which may be obscured by gene interactions and modified by environmental factors.

Analyzing the action of single genes in polygenic traits is still out of the question because each locus, aside from contributing only partially to the genotype, undergoes variability deriving from other factors, both genetic (penetration, phenocopies, incomplete co-segregation, parent-specific imprinting) and environmental, which make separation of these effects problematic.

Several statistical strategies have been set up, based on the concept that on the whole some genes have a greater action than others in determining a trait; it would therefore be possible to separate their quantitative effect and identify associated markers which may justify up to 10 or 20% of the trait's variability.

Anyway, the detection of major genes and of the genomic markers associated with them is a very promising research goal for reinforcing selective progress in quantitative traits by means of *Marker Assisted Selection* (MAS). Genetic linkage maps can provide the basis for determining *Quantitative Trait Loci* (QTL) and furthering genetic progress, especially for traits which may be difficult or expensive to measure such as carcass or meat quality (Stone et al., 1999).

Current research aims to establish a unified model of estimation for each QTL, in which various regions of the genome are assigned an importance proportional to the QTL variance which they explain. A

unified model would permit better examination of additive effects, tracing alleles combinations, estimation and prediction of non-additive genetic effects such as heterosis and consanguinity (Haley & Wischer, 1998). A methodology applied to the analysis of variability, which can also be applied to the study of QTL, was formulated by Ciampolini et al. (1995, 2000).

MATERIALS AND METHODS

Somatic measurements

The study was conducted on 47 young double-muscled bulls in a performance test. The morpho-functional evaluations as established by the Breed Standard were performed by ANABORAPI (*Associazione Nazionale Bovini di Razza Piemontese*).

DNA analysis

DNA was purified from 20-ml samples of peripheral blood using the method described by Jeanpierre (1987). The PCR reactions and the procedures for determining the genotypes of the microsatellites were performed according to the methodology described by Vaiman et al. (1994). For this study 20 microsatellites were analyzed; 17 of these were produced in the INRA laboratories (Vaiman et al., 1994) and the rest by Steffen et al. (1993). Information concerning the 20 microsatellites is shown in Table I.

Statistical analysis

The average values of the somatic measurements of subjects carrying every allele of each microsatellite were calculated; these were compared statistically with the average values of subjects not carrying the allele, and the significance of the difference was estimated using the J.M.P. computer programme (1996). The subjects carrying alleles with positive or negative implications for meat traits were grouped in sub-populations and the biosys computer package (Swofford & Selander, 1989) was applied for the calculation of allele frequencies, Hardy-Weinberg proportions, excess or deficiency of heterozygotes and genetic distance according to Cavalli Sforza and Edwards (1967).

The genetic similarities between and within populations were esti-

Tab. I. Analysed microsatellites and their polymorphism.				
Marker	Locus	Chromosome	Bibliography	Numbers of alleles
INRA 5	D12S4	12	Vaiman et al., 1994	5
INRA 6	D3S9	3	Vaiman et al., 1994	6
INRA 11	D1S6	1	Vaiman et al., 1994	10
INRA 13	D16S10	16	Vaiman et al., 1994	9
INRA 16	D27S20	27	Vaiman et al., 1994	10
INRA 23	D3S10	3	Vaiman et al., 1994	11
INRA 25	D17S6	17	Vaiman et al., 1994	8
INRA 27	D27S16	27	Vaiman et al., 1994	6
INRA 31	D21S12	21	Vaiman et al., 1994	6
INRA 32	D11S9	11	Vaiman et al., 1994	9
INRA 35	D16S11	16	Vaiman et al., 1994	7
INRA 37	D4S26	4	Vaiman et al., 1994	12
INRA 50	D15S5	15	Vaiman et al., 1994	10
INRA 53	D7S6	7	Vaiman et al., 1994	5
INRA 63	D18S5	18	Vaiman et al., 1994	7
INRA 64	D23S15	23	Vaiman et al., 1994	6
INRA 72	D4S11	4	Vaiman et al., 1994	10
ETH 131	D21S4	21	Steffen et al., 1993	13
ETH 152	D5S1	5	Steffen et al., 1993	7
ETH 225	D9S1	9	Steffen et al., 1993	5

mated according to the method of Ciampolini et al., (1995) based on the *Individual Multilocus Genotype* (IMG). Each subject was defined according to its own multilocus genotype (in our case, 20 microsatellite loci) consisting of a series of 40 alleles. In order to estimate the genetic similarity between two individuals, or groups of individuals, the proportion (P) of shared alleles (A) in relation to the 2L possibilities (L = number of loci considered) was calculated.

Genetic similarity was measured by $P = A/2L$. The similarities calculated for each pair of subjects were averaged in order to obtain similarity values between breeds or subpopulations. To estimate the similarity (or the genetic distance) between breeds or subpopulations the average values of the similarities between each subject of a group and each subject of the comparison group were calculated.

RESULTS

The study revealed numerous microsatellites with alleles significantly linked to positive or negative meat production traits. The most interesting of the 20 cases we studied were microsatellites INRA 5 (locus D12S4), INRA 11 (locus D1S6), INRA 16 (locus D27S16), INRA 64 (locus D23S15), ETH 131 (locus D21S4), with alleles present in subjects with greater somatic development, and obviously different alleles present in less-developed subjects.

In order to point out the differences on a somatic level, we calculated the average values of somatic measurements in subjects carrying the most significant and interesting alleles (both positive and negative). These values, compared to the average values of the measurements of the non-carrier individuals, at times show significantly different values ($P < 0.01$ or $P < 0.05$).

In particular, the INRA 5 microsatellite (chromosome 12) presents Allele 4 in those subjects with most of the measurements, as well as a live weight at 250 and 300 days, greater than that of non-carrier subjects ($P < 0.01$). On the other hand Allele 3 was present in many less-developed subjects (but for $P < 0.05$). As shown in Table II, the differences between the subjects carrying Allele 4 and those with Allele 3 were particularly noticeable, arriving at a 2-cm difference in height and a 20-kg weight difference.

Tab. II. Somatic measurements of animals evidencing alleles at locus D12S4 (INRA 5) associated^a with beef characters.

	Subjects with allele 4		Other subjects		Subjects with allele 3		Other subjects	
	Mean	SD ^b	Mean	SD	Mean	SD	Mean	SD
Height at withers, cm	118.81*	1.91	117.31	1.87	116.77*	1.78	118.53	1.96
Height at pelvis, cm	126.60**	1.72	125.01	1.58	124.76*	1.42	126.25	1.81
Depth of chest, cm	61.39**	1.04	60.44	0.95	60.28*	0.86	61.18	1.09
Body length, cm	142.49**	2.90	139.81	2.66	139.39*	2.39	141.89	3.05
Chest length, cm	77.21**	0.98	76.30	0.90	76.15*	0.81	77.01	1.04
Length of rump, cm	50.49**	0.79	49.76	0.73	49.64*	0.66	50.32	0.83
Width of brisket, cm	40.43	0.88	39.96	0.79	39.80	0.71	40.34	0.87
Width of chest, cm	43.82	0.85	43.63	0.64	43.45	0.52	43.81	0.81
Fore width of rump, cm	43.50**	0.96	42.61	0.88	42.47*	0.80	43.30	1.01
Medium width of rump, cm	45.71*	1.01	45.07	0.96	44.97	0.94	45.57	1.03
Hind width of rump, cm	38.96	0.89	38.47	0.83	38.39	0.82	38.85	0.90
Length of head, cm	43.80	1.49	43.12	0.99	43.57	0.97	43.55	1.43
Crest girth, cm	182.68	2.95	181.40	2.69	181.20	2.73	182.39	2.92
Fore cannon girth, cm	18.95*	0.33	18.68	0.31	18.63	0.28	18.89	0.35
Buttock girth, cm	140.69	6.42	140.08	4.03	139.50	3.32	140.61	5.97
Weight at 150 d, kg	161.71	5.90	160.07	5.72	157.94	6.93	161.68	5.53
Weight at 250 d, kg	322.51**	22.04	303.46	18.87	302.51	17.23	317.91	22.94
Weight at 350 d, kg	437.75**	18.63	420.56	17.12	417.82*	15.38	433.93	19.60
Daily gain from 150 to 250 d, kg	1.360	0.190	1.362	0.130	1.400	0.160	1.354	0.170
Daily gain from 150 to 350 d, kg	1.391	0.140	1.372	0.110	1.418	0.110	1.378	0.140
Daily gain from 250 to 350 d, kg	1.433	0.220	1.384	0.170	1.442	0.180	1.410	0.210
Referees score	5.617	0.760	5.800	0.810	5.669	0.790	5.688	0.790

^a By convention; * designates $P < 0.05$ and ** designates $P < 0.01$.

^b SD designates standard deviation.

The INRA 11 microsatellite (chromosome 1) presents Allele 1 in subjects that are significantly superior to the rest of the population in growth, hindquarter development and evaluation of the referees. Instead Allele 6 characterizes subjects with lower growth rate, larger head and negative referee evaluation (Tab. III). In particular, the growth rate of subjects with Allele 1 surpasses that of subjects with Allele 6 by more than 12%.

The INRA 16 microsatellite (chromosome 27) was present with Alleles 7 and 8 in animals with good hindquarter development and with Allele 9 in the subjects with greater growth and more positive referee evaluation (Tab. IV); instead Allele 10 determines lower growth rate (13% less than Allele 9).

The case of the INRA 64 microsatellite (chromosome 23) is very unusual; it has no alleles with positive significance but it is interesting due to Allele 3, present in subjects with a significantly reduced development ($P < 0.05$ or $P < 0.01$, depending on the measurements) of nearly all parameters and even $P < 0.001$ for chest width (Tab. V).

The ETH 131 microsatellite (chromosome 21) repropose the reduced somatic development with the allele 10 but characterizes good growth with allele 8 and positive referee evaluation with allele 7 (Tab. VI).

Genomic formula of each subject was examined in order to obtain information about the number of alleles significantly associated with either positive or negative morphological expressions regarding meat production. This led to dividing the subjects into three subpopulations: one consisting of 12 individuals with a large number of positive alleles; another, of 7 subjects, with a marked prevalence of negative alleles. the last group of subjects (the largest) presents nearly the same number of positive and negative alleles (data not shown).

The allele frequencies were calculated and the genetic distances estimated for the two subpopulations that were farthest apart. All methods (Nei, 1972; Edwards, 1971, 1974; Cavalli Sforza & Edwards, 1967) indicated that the two groups of subjects were significantly distant from each other, with a value of 0.365 (Tab. VII) according to the arc distance method of Cavalli Sforza and Edwards (1967).

Hardy-Weinberg proportions (Table VIII) were not respected by any microsatellite of the positive subjects nor by three of the negative

Tab. III. Somatic measurements of animals evidencing alleles at locus D1S6 (INRA 11) associated ^a with beef characters.									
	Subjects with allele 1		Other subjects		Subjects with allele 6		Other subjects		
	Mean	SD ^b	Mean	SD	Mean	SD	Mean	SD	
Height at withers, cm	118.29	1.90	118.27	2.06	117.76	2.13	118.72	1.84	
Height at pelvis, cm	126.03	1.75	126.03	1.86	125.52	1.70	126.48	1.84	
Depth of chest, cm	61.05	1.06	61.05	1.12	60.74	1.02	61.32	1.11	
Body length, cm	141.52	2.96	141.52	3.13	140.66	2.86	142.28	3.11	
Chest length, cm	76.88	1.00	76.88	1.06	76.59	0.97	77.14	1.06	
Length of rump, cm	50.22	0.81	50.22	0.85	49.99	0.78	50.43	0.85	
Width of brisket, cm	40.43	0.49	40.23	0.92	40.17	0.91	40.34	0.83	
Width of chest, cm	43.90	0.45	43.73	0.83	43.74	0.76	43.77	0.81	
Fore width of rump, cm	43.18	0.98	43.18	1.04	42.89	0.95	43.43	1.03	
Medium width of rump, cm	45.71	0.59	45.44	1.09	45.37	1.07	45.57	1.01	
Hind width of rump, cm	39.00	0.46	38.74	0.95	38.72	0.93	38.84	0.88	
Length of head, cm	44.00	1.91	43.48	1.26	43.27	1.08	43.80	1.55	
Crest girth, cm	183.04	1.32	182.07	3.08	182.16	2.98	182.26	2.89	
Fore cannon girth, cm	18.84	0.34	18.88	0.35	18.80	0.32	18.92	0.36	
Buttock girth, cm	144.71*	6.68	139.66	5.25	139.83	5.54	141.00	5.96	
Weight at 150 d, kg	159.98	6.60	161.32	5.76	159.87	5.87	162.22	5.68	
Weight at 250 d, kg	316.88	20.46	315.40	23.31	310.30	19.85	320.30	24.39	
Weight at 350 d, kg	431.53	19.02	431.53	20.12	426.00	18.38	436.40	19.99	
Daily gain from 150 to 250 d, kg	1.534**	0.100	1.330	0.160	1.342	0.130	1.378	0.200	
Daily gain from 150 to 350 d, kg	1.548***	0.120	1.355	0.110	1.341*	0.100	1.422	0.150	
Daily gain from 250 to 350 d, kg	1.565*	0.220	1.389	0.190	1.340*	0.160	1.481	0.220	
Referees score	6.278*	0.760	5.596	0.750	5.430*	0.710	5.919	0.780	

^a By convention; * designates P < 0.05, ** designates P < 0.01 and *** designates P < 0.001.
^b SD designates standard deviation.

Tab. IV. Somatic measurements of animals evidencing alleles at locus D27S20 (INRA 16) associated^a with beef characters.

	Subjects with allele 8		Other subjects		Subjects with allele 9		Other subjects	
	Mean	SD ^b	Mean	SD	Mean	SD	Mean	SD
Height at withers, cm	118.31	1.28	118.26	2.15	118.2	1.63	118.28	2.09
Height at pelvis, cm	125.92	1.01	126.05	1.96	125.9	1.15	126.05	1.93
Depth of chest, cm	60.98	0.61	61.06	1.18	60.97	0.69	61.06	1.16
Body length, cm	141.34	1.71	141.56	3.30	141.32	1.94	141.56	3.25
Chest length, cm	76.82	0.58	76.89	1.12	76.81	0.66	76.89	1.10
Length of rump, cm	50.17	0.47	50.23	0.90	50.17	0.53	50.23	0.89
Width of brisket, cm	40.15	0.52	40.28	0.93	40.61	0.61	40.20	0.90
Width of chest, cm	43.72	0.33	43.76	0.85	44.08	0.69	43.70	0.79
Fore width of rump, cm	43.12	0.57	43.19	1.10	43.11	0.64	43.19	1.08
Medium width of rump, cm	45.27	0.76	45.52	1.08	45.95	0.54	45.39	1.08
Hind width of rump, cm	38.59	0.68	38.82	0.93	39.25	0.46	38.70	0.93
Length of head, cm	43.75	0.49	43.51	0.22	43.29	0.76	43.60	1.45
Crest girth, cm	181.55	2.30	182.35	3.02	183.98	1.43	181.91	2.99
Fore cannon girth, cm	18.82	0.19	18.88	0.38	18.82	0.24	18.88	0.36
Buttock girth, cm	144.38*	5.34	139.59	5.52	140	5.48	140.58	5.86
Weight at 150 d, kg	162.80	6.41	160.77	5.74	158.43	5.59	161.59	5.81
Weight at 250 d, kg	312.30	13.81	316.30	24.21	317.04	13.14	315.37	24.11
Weight at 350 d, kg	430.35	10.99	431.78	21.21	430.23	12.48	431.76	20.88
Daily gain from 150 to 250 d, kg	1.37	0.17	1.36	0.17	1.495*	0.09	1.34	0.17
Daily gain from 150 to 350 d, kg	1.38	0.18	1.39	0.12	1.477*	0.12	1.37	0.13
Daily gain from 250 to 350 d, kg	1.38	0.31	1.42	0.18	1.453	0.22	1.41	0.21
Referees score	5.80	0.71	5.66	0.80	6.32*	0.83	5.57	0.72

Tab. IV. Somatic measurements of animals evidencing alleles at locus D27S20 (INRA 16) associated^a with beef characters. (*continued*)

	Subjects with allele 7		Other subjects		Subjects with allele 10		Other subjects	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height at withers, cm	117.50	1.65	118.45	2.07	118.33	2.38	118.23	1.82
Height at pelvis, cm	125.80	1.36	126.08	1.93	126.19	2.15	125.94	1.64
Depth of chest, cm	60.91	0.82	61.08	1.16	61.14	1.30	60.99	0.99
Body length, cm	141.14	3.29	141.61	3.25	141.79	3.63	141.37	2.77
Chest length, cm	76.75	0.78	76.91	1.10	76.97	1.23	76.83	0.94
Length of rump, cm	50.12	0.62	50.25	0.89	50.30	0.99	50.18	0.76
Width of brisket, cm	40.16	0.71	40.29	0.91	40.18	1.01	40.31	0.79
Width of chest, cm	43.55	0.61	43.80	0.82	43.64	1.01	43.82	0.63
Fore width of rump, cm	43.05	0.76	43.21	1.08	43.27	1.21	43.13	0.92
Medium width of rump, cm	45.55	0.87	45.46	1.08	45.40	1.12	45.52	1.00
Hind width of rump, cm	38.87	0.76	38.76	0.93	38.69	0.97	38.83	0.86
Length of head, cm	43.78	1.56	43.50	1.33	43.47	1.50	43.60	1.30
Crest girth, cm	182.63	2.52	182.12	3.00	181.82	3.17	182.44	2.77
Fore cannon girth, cm	18.83	0.26	18.88	0.37	18.94	0.39	18.83	0.32
Buttock girth, cm	141.29**	4.79	140.34	5.97	139.00	5.79	141.33	5.66
Weight at 150 d, kg	156.50	5.68	162.21	5.38	161.55	5.59	160.88	5.46
Weight at 250 d, kg	317.82	16.69	315.10	24.07	317.25	26.34	314.69	20.81
Weight at 350 d, kg	429.10	14.69	432.11	20.90	433.27	23.30	430.55	17.80
Daily gain from 150 to 250 d, kg	1.45	0.13	1.34	0.17	1.28**	0.19	1.41	0.14
Daily gain from 150 to 350 d, kg	1.39	0.13	1.38	0.13	1.34	0.10	1.41	0.14
Daily gain from 250 to 350 d, kg	1.30	0.20	1.44	0.20	1.42	0.18	1.41	0.22
Referees score	5.66	0.43	5.69	0.85	5.41	0.83	5.85	0.70

^a By convention; * designates $P < 0.05$ and ** designates $P < 0.01$.^b SD designates standard deviation.

Tab. V. Somatic measurements of animals evidencing alleles at locus D23S15 (INRA 64) associated^a with beef characters.

	Subjects with allele 1		Other subjects	
	Mean	SD ^b	Mean	SD
Height at withers, cm	116.42**	1.37	118.77	1.88
Height at pelvis, cm	124.89*	1.39	126.33	1.82
Depth of chest, cm	60.36*	0.84	61.23	1.10
Body lenght, cm	139.61*	2.34	142.04	3.07
Chest lenght, cm	76.23*	0.79	77.05	1.04
Lenght of rump, cm	49.70*	0.64	50.36	0.84
Width of brisket, cm	39.51**	0.60	40.46	0.82
Width of chest, cm	43.07***	0.46	43.94	0.75
Fore width of rump, cm	42.55*	0.78	43.35	1.02
Medium width of rump, cm	44.75*	0.82	45.67	1.00
Hind width of rump, cm	38.16*	0.71	38.95	0.87
Lenght of head, cm	43.90	1.10	43.46	1.43
Crest girth, cm	180.32*	2.32	182.73	2.85
Fore cannon girth, cm	18.64*	0.27	18.93	0.34
Buttock girth, cm	140.89	5.23	140.39	5.95
Weight at 150 d, kg	156.60**	4.74	162.34	5.53
Weight at 250 d, kg	305.75	18.47	318.28	23.22
Weight at 350 d, kg	419.26*	15.07	434.85	19.72
Daily gain from 150 to 250 d, kg	1.372	0.120	1.358	0.180
Daily gain from 150 to 350 d, kg	1.374	0.110	1.387	0.140
Daily gain from 250 to 350 d, kg	1.377	0.200	1.425	0.210
Referees score	5.551	0.790	5.722	0.780

^a By convention; * designates $P < 0.05$, ** designates $P < 0.01$ and *** designates $P < 0.001$.^b SD designates standard deviation.

Tab. VI. Somatic measurements of animals evidencing alleles at locus D21S4 (ETH 131) associated^a with beef characters.

	Subjects with allele 8		Other subjects		Subjects with allele 10		Other subjects	
	Mean	SD ^b	Mean	SD	Mean	SD	Mean	SD
Height at withers, cm	117.98	2.08	118.32	2.03	116.88*	1.60	118.51	2.00
Height at pelvis, cm	126.07	1.78	126.02	1.85	124.61*	1.31	126.27	1.80
Depth of chest, cm	61.07	1.07	61.04	1.12	60.19*	0.79	61.20	1.08
Body length, cm	141.59	3.00	141.51	3.12	139.13*	2.21	141.94	3.03
Chest length, cm	76.90	1.02	76.87	1.06	76.07*	0.75	77.02	1.03
Length of rump, cm	50.24	0.82	50.22	0.85	49.57*	0.60	50.34	0.83
Width of brisket, cm	40.33	0.75	40.25	0.90	39.58*	0.69	40.38	0.85
Width of chest, cm	43.73	0.52	43.76	0.82	43.35	0.57	43.83	0.80
Fore width of rump, cm	43.20	1.00	43.18	1.04	42.38*	0.73	43.32	1.01
Medium width of rump, cm	45.71	0.98	45.44	1.05	44.56**	0.84	45.64	0.99
Hind width of rump, cm	39.00	0.83	38.74	0.91	38.00**	0.73	38.92	0.85
Length of head, cm	43.71	0.95	43.53	1.43	43.00	1.15	43.65	1.39
Crest girth, cm	183.02	2.65	182.07	2.95	179.84*	2.41	182.63	2.80
Fore cannon girth, cm	18.94	0.26	18.86	0.36	18.57**	0.25	18.93	0.34
Buttock girth, cm	144.50	4.64	139.83	5.69	138.00	5.89	141.00	5.67
Weight at 150 d, kg	157.94	7.27	161.68	5.46	161.28	4.88	161.09	6.04
Weight at 250 d, kg	319.72	21.38	314.90	23.12	296.75*	15.35	318.92	22.29
Weight at 350 d, kg	431.99	19.32	431.45	20.07	416.16*	14.20	434.22	19.48
Daily gain from 150 to 250 d, kg	1.490*	0.100	1.338	0.170	1.232*	0.160	1.383	0.160
Daily gain from 150 to 350 d, kg	1.476*	0.120	1.368	0.130	1.330	0.150	1.394	0.130
Daily gain from 250 to 350 d, kg	1.458	0.220	1.408	0.210	1.461	0.200	1.407	0.210
Referees score	5.919	0.630	5.643	0.800	5.704	0.920	5.681	0.760

^a By convention; * designates $P < 0.05$ and ** designates $P < 0.01$.^b SD designates standard deviation.

Table VII. Genetic distances between and within furthestmost subpopulations

Methods	between POS ^a and NEG ^b	Genetic distances	
		within POS	within NEG
Nei (1972)	0.211	-	-
Edwards (1971, 1974)	0.424	-	-
Cavalli-Sforza and Edwards (1967)	0.365 ^c	-	-
Cavalli-Sforza and Edwards (1967)	0.373 ^d	-	-
Ciampolini et al., (1995)	0.364	0.359	0.43

^a POS designates positive subpopulation; ^b NEG designates negative subpopulation; ^c 0.365 = chord distance; ^d 0.373 = arc distance.

subjects. Also, few microsatellites were in clear disequilibrium. A greater significance for the coefficients of heterozygotes deficiency confirms the tendency toward homozygosis.

The calculation of genetic similarity performed according to the method of Ciampolini et al. (1995) indicates a great homogeneity in the group of subjects with alleles less implicated in meat traits (coefficient of similarity within the population equivalent to 0.430) compared to the subjects with positive alleles (similarity coefficient = 0.359, Table VIII).

DISCUSSION

In a previous study (Ciampolini et al., in press) we undertook to investigate the relationships between the morpho-functional typology of Piemontese beef cattle and the Individual Multilocus Genotype (Ciampolini et al, 1995) determined by means of DNA microsatellites. This work continues our investigation of the same problem, analyzing the relationships between DNA microsatellites (and their alleles) and the morpho-functional traits of young Piemontese bulls.

Similarly, in the same breed, several authors (Charlier et al., 1995; Grobet et al., 1997), by means of linkage analysis, have demonstrated the relation between double muscling trait and locus *mh* (muscular hypertrophy), located on BTA 2. A positional candidate approach sub-

Tab. VIII. Hardy-Weinberg proportions and coefficients for heterozygotes deficiency for subjects with a high number of alleles associated^a with positive or negative beef characters.

Marker	Hardy- Weinberg proportions ^b			Coefficients for heterozygote deficiency ^c .		
	POS ^d	NEG ^e	UNC ^f	POS	NEG	UNC
INRA 5	0.137	0.617	0.078	0.325 **	0.322 **	-0.024
INRA 6	0.900	0.955*	0.007	0.103	0.114	-0.019
INRA 11	0.248	0.955*	0.029	0.073	0.114	-0.070
INRA 13	0.382	0.210	0.863	-0.275 **	-0.487 ***	-0.082
INRA 16	0.726	0.738	0.640	0.068	0.379 **	-0.153
INRA 23	0.931	0.580	0.362	0.041	0.000	-0.154
INRA 25	0.020*	0.699	0.748	0.045	0.000	-0.089
INRA 27	0.118	0.843	0.683	-0.352 **	-0.133	-0.210
INRA 31	0.759	0.931	0.137	0.173	0.219 *	-0.057
INRA 32	0.844	0.993**	0.091	0.056	0.040	-0.149
INRA 35	0.253	0.004**	0.168	-0.195 *	-0.629 ***	-0.377
INRA 37	0.000***	0.160	0.000	-0.203 *	-0.226 *	-0.429
INRA 50	0.059	0.160	0.207	-0.248 *	-0.015	-0.239
INRA 53	0.562	0.582	0.029	0.070	-0.015	-0.114
INRA 63	0.684	0.786	0.002	-0.241 *	0.020	-0.107
INRA 64	0.019*	0.042*	0.509	-0.418 ***	-0.278 **	-0.262
INRA 72	0.753	0.697	0.000	-0.072	-0.257 **	-0.004
ETH 131	0.846	0.020	*0.073	0.095	-0.388 ***	0.034
ETH 152	0.241	0.740	0.746	0.000	-0.058	0.200
ETH 225	0.197	0.216	0.007	-0.037	0.264 *	-0.033

^a By convention; * designates P < 0.05, ** designates P < 0.01 and *** designates P < 0.001.
^b 0.000 = full disequilibrium; 1.000 = full equilibrium; ^c values below zero = heterozygotes deficiency; ^d POS = positive sobpopulation;
^e NEG = negative subpopulation; ^f UNC = uncertain subpopulation.

sequently allowed to identifying an exon 3 missense mutation of myostatin gene as the cause of double muscling in Piemontese cattle breed (Kambadur et al., 1997; McPherron et al., 1997; Smith et al., 1997).

In this study many microsatellites (18 of 20) presented alleles which were significantly linked to somatic measurements more or less relevant to meat production; however only five markers merit further attention since they are present with one allele in more developed individuals and with other alleles in less-developed subjects.

In spite of the aforementioned doubts and uncertainties attending the association of a quantitative trait, such as a somatic measurement or weight gain, to a single marker, the presence of many traits with variability linked to the same alleles appears to confirm that it is no accidental phenomenon. Therefore it also confirms (statistically, as well) the real distance of the population of subjects carrying that allele from those which do not.

Although we propose further investigation of the problem, the results of this study confirm the validity of our methodology, which allowed us to identify several microsatellites which appear to be associated with the production performance of beef cattle. This was reflected in:

- Significant differences between the subpopulation of subjects carrying a certain allele and the subpopulation of carriers of other alleles.
- The consistency between a certain genotype and the phenotypical results (positive or negative) for meat production traits.
- The genetic similarity between subjects corresponding less to the meat typology and the dissimilarity between the ones corresponding more closely to it.

This last observation confirms a previous study's results (Ciampolini et al., 2001) attributed to the persistence of a base population which remains homogeneous; at the same time, however, the population seems to contain certain "peaks" of advanced selection.

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